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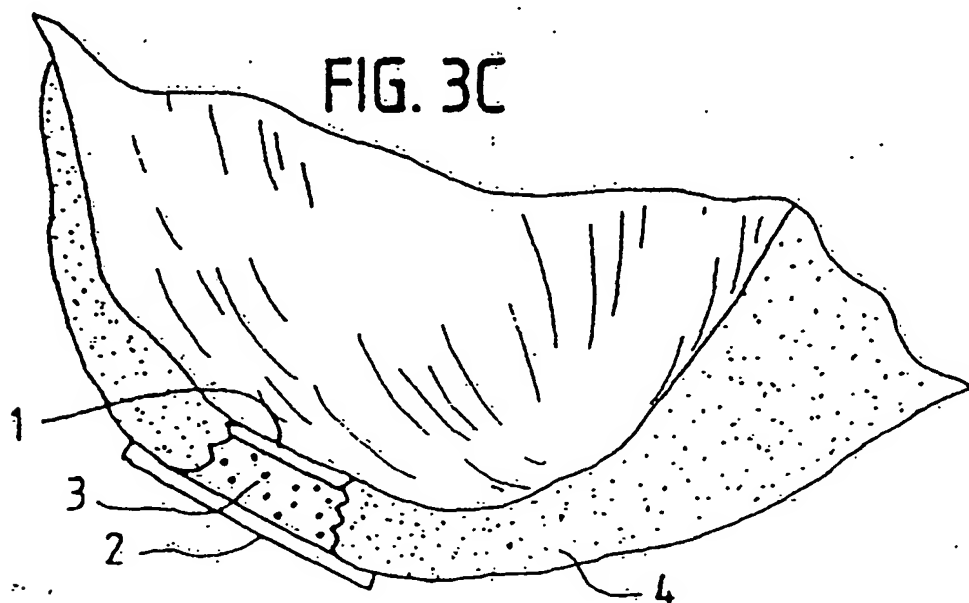
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(54) **Method, instruments and kit for autologous transplantation**

(57) Method for the effective transplantation of chondrocytes/cartilage to an articular joint surface defect is taught,
as well as a description of certain instruments and kit for practicing the invention.



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Description

Field of the Invention

[0001] The instant invention concerns the field of chondrocyte transplantation, bone and cartilage grafting, healing, joint repair and the prevention of arthritic pathologies. In particular methods for the preparation of the graft site, instruments for such preparation and for the autologous transplantation of cells to the prepared graft site.

Background of the Invention

[0002] More than 500,000 arthroplastic procedures and total joint replacements are performed each year in the United States. Approximately the same number of similar procedures are performed in Europe. Included in these numbers are about 90,000 total-knee replacements and around 50,000 procedures to repair defects in the knee per year in Europe. The number of procedures are essentially the same in the U. S. (In: Praemer A., Furner S., Rice, D.P., Musculoskeletal conditions in the United States, American Academy of Orthopaedic Surgeons, Park Ridge, 111., 1992, 125). A method for regeneration-treatment of cartilage would be most useful, and could be performed at an earlier stage of joint damage, thus reducing the number of patients needing artificial joint replacement surgery. With such preventative methods of treatment, the number of patients developing osteoarthritis would also decrease.

[0003] Techniques used for resurfacing the cartilage structure in joints have mainly attempted to induce the repair of cartilage using subchondral drilling, abrasion and other methods whereby there is excision of diseased cartilage and subchondral bone, leaving vascularized cancellous bone exposed (Insall, J., *Clin. Orthop.* 1974, 101, 61; Ficat R.P. et al, *Clin Orthop.* 1979, 144, 74; Johnson L.L., In: Operative Arthroscopy, McGinty J.B., Ed., Raven Press, New York, 1991, 341).

[0004] Coon and Cahn (*Science* 1966, 153, 1116) described a technique for the cultivation of cartilage synthesizing cells from chick embryo somites. Later Cahn and Lasher (*PNAS USA* 1967, 58, 1131) used the system for analysis of the involvement of DNA synthesis as a prerequisite for cartilage differentiation. Chondrocytes respond to both EFG and FGF by growth (Gospodarowicz and Mescher, *J. Cell Physiology* 1977, 93, 117), but ultimately lose their differentiated function (Benya et al., *Cell* 1978, 15, 1313). Methods for growing chondrocytes were described and are principally being used with minor adjustments by Brittberg, M. et al. (*New Engl. J. Med.* 1994, 331, 889). Cells grown using these methods were used as autologous transplants into knee joints of patients. Additionally, Kolettas et al. (*J. Cell Science* 1995, 108, 1991) examined the expression of cartilage-specific molecules such as collagens and proteoglycans under prolonged cell culturing. They found that despite

morphological changes during culturing in monolayer cultures (Aulthouse, A. et al., *In Vitro Cell Dev. Biol.*, 1989, 25, 659; Archer, C. et al., *J. Cell Sci.* 1990, 97, 361; Hänselmann, H. et al., *J. Cell Sci.* 1994, 107, 17; Bonaventure, J. et al., *Exp. Cell Res.* 1994, 212, 97), when compared to suspension cultures grown over agarose gels, alginate beads or as spinner cultures (retaining a round cell morphology) tested by various scientists did not change the chondrocyte - expressed markers such as types II and IX collagens and the large aggregating proteoglycans, aggrecan, versican and link protein did not change (Kolettas, E. et al., *J. Cell Science* 1995, 108, 1991).

[0005] The articular chondrocytes are specialized mesenchymal derived cells found exclusively in cartilage. Cartilage is an avascular tissue whose physical properties depend on the extracellular matrix produced by the chondrocytes. During endochondral ossification chondrocytes undergo a maturation leading to cellular hypertrophy, characterized by the onset of expression of type X collagen (Upholt, W.B. and Olsen, R.R., In: Cartilage Molecular Aspects (Hall, B & Newman, S. Eds.) CRC Boca Raton 1991, 43; Reichenberger, E. et al., *Dev. Biol.* 1991, 148, 562; Kirsch, T. et al., *Differentiation*, 1992, 52, 89; Stephens, M. et al., *J. Cell Sci.* 1993, 103, 1111).

[0006] Excessive degradation of type II collagen in the outer layers or articular surfaces of joints is also caused by osteoarthritis. The collagen network is accordingly weakened and subsequently develops fibrillation whereby matrix substances such as proteoglycans are lost and eventually displaced entirely. Such fibrillation of weakened osteoarthritic cartilage can reach down to the calcified cartilage and into the subchondral bone (Kempson, G.E. et al., *Biochim. Biophys. Acta* 1976, 428, 741; Roth, V. and Mow, V.C., *J. Bone Joint Surgery*, 1980, 62A, 1102; Woo, S.L.-Y. et al., in Handbook of Bioengineering (R. Skalak and S. Chien eds.), McGraw-Hill, New York, 1987, pp. 4.1-4.44).

[0007] Descriptions of the basic development, histological and microscopic anatomy of bone, cartilage and other such connective tissues can be found for example in Wheeler, Burkitt and Daniels, Functional Histology, 2nd Edition, (Churchill Livingstone, London, 1987, Chp. 4). Descriptions of the basic histological anatomy of defects in bone, cartilage and other connective tissue can be found for example in Wheeler, Burkitt, Stevens and Lowe, Basic Histopathology, (Churchill Livingstone, London, 1985, Chp. 21).

[0008] Despite the advances in cultivating chondrocytes, and manipulating bone and cartilage, there has not been great success with the attempts to transplant cartilage or chondrocytes for the repair of damaged articulating surfaces. The teachings of the instant invention provide for effective and efficient means of promoting the transplantation of cartilage and/or chondrocytes into a defect in an articulating joint or other cartilage covered bone surface, whereby cartilage is regenerated to

fix the defect. The instant invention also provides for surgical instruments which are designed prepare the graft site so as to facilitate the efficient integration of grafted material to the graft site.

Brief Summary of the Invention

[0009] The instant invention provides a method for the effective treatment of articulating joint surface cartilage by the transplantation of chondrocytes in a suitable matrix, to a surface to be treated, with a hemostatic barrier and a cell-free covering-patch comprising; first placing a hemostatic barrier proximal to the surface to be treated, placing chondrocytes in a suitable matrix upon the surface to be treated distal to the hemostatic barrier, covering the surface to be treated with a cell-free covering-patch. A hemostatic barrier, as will be further described below, is a barrier which inhibits or prevents the penetration of vascularizing cells and tissue into the grafted material. In particular, the instant method provides for a hemostatic barrier that is a resorbable, semi-permeable material which inhibits or prohibits vascular infiltration through the barrier. In one embodiment the hemostatic barrier contains collagen. Cell-free, is used herein as in the art, and means a material that is substantially free from intact cells which are capable of further cell division, promulgation or biological activity. In a preferred embodiment, a cell-free material is free from all intact nucleated cells. In one embodiment, the instant method encompasses the use of a cell-free covering patch which contains a semi-permeable collagen matrix. In one preferred embodiment of the method, the porous surface of the cell-free covering-patch is directed towards the implant material.

[0010] The instant invention further provides for the autologous transplantation of collagen or chondrocytes to a graft site, wherein the graft site has first been prepared by surgical manipulation to better accept the grafted material. In one embodiment, the graft site is sculpted such that the walls of the graft site are contoured in an undulating pattern such that the grafted material, when placed within the graft site and expanded to contact the graft site wall, there will be resistance against removal or expulsion of the entire graft from the graft site. The instant invention further provides for surgical instruments designed to sculpt the graft site as taught by the method of the invention.

[0011] The invention further provides for a kit for cartilage and/or chondrocyte transplantation onto the surface of an articular joint wherein said kit comprises a hemostatic barrier, cell-free semi-permeable covering-patch, and organic glue. In a further embodiment, the kit can optionally further provide one or more surgical instruments which can be used to sculpt the graft site in accordance with the methods of the instant invention.

Brief Description of the Drawings

[0012] The present invention will be better understood by examining the following figures which illustrate certain properties of the instant invention wherein:

Figure 1A is a drawing showing a typical articulating end of a bone. Typically, the bone material is covered on the articulating surface with a cartilaginous. Figure 1B shows an example of where a defect or injury to the cartilaginous cap occurs (gap in cartilage); and such a defect can be treated directly, enlarged slightly, or sculpted to accept the grafted material by surgical procedures prior to treatment.

Figure 1C shows how the hemostatic barrier (numbered 1) is placed within the defect in the cartilage cap to inhibit or prevent vascularization into regenerating cartilage, from the underlying bone. The chondrocytes to be implanted into the defect cavity are then layered on top of the hemostatic barrier.

Figure 2 is a drawing showing the treated defect (gap in cartilage) in the cartilaginous cap covered by a cell-free semi-permeable material (numbered 2) which is used to form a cap/patch or bandage over the defect site. This cap is fixed in place, either sutured to the edge of the cavity into healthy cartilage, or otherwise attached. This cap is covering the defective area of the joint into which the cultured chondrocytes/cartilage transplant has been placed, or will be placed under the partially attached cap.

Figure 3A is a diagram illustrating the differential response to compression and shearing forces by harder and softer cartilage with subsequent zone of demarcation.

Figure 3B illustrates the graft site, after the defect has been sculpted to have undulating walls.

Figure 3C illustrates the sculpted graft site with the hemostatic barrier (1), transplanted material (3), and cell-free covering-patch (2) in place within the articular surface cartilage (4).

Figure 4A illustrates one embodiment of the surgical device of the instant invention showing cutting teeth (5) and protruding placement pin (6). The cross-section illustrations to the right show two possible configurations of the cutting blades.

Figure 4B illustrates a second embodiment of the surgical device of the instant invention.

Figure 5 is a diagram illustrating the modified differential response to compression and shearing forces by harder cartilage and softer cartilage after sculpting the graft site.

Figure 6A is an MRI image of a pig knee showing cartilage defect in left (medial) condyle.

Figure 6B is an MRI image of the same pig knee three months after treatment.

Detailed Description of the Invention

[0013] This invention concerns the use of certain products that inhibit the formation of vascular tissue, for instance such as capillary loops projecting into the cartilage being established, during the process of autologous transplantation of chondrocytes into defects in the cartilage. The formation of vascular tissue from the underlying bone will tend to project into the new cartilage to be formed leading to appearance of cells other than the mesenchymal specialized chondrocytes desired.

[0014] The contaminating cells introduced by the vascularization may give rise to encroachment and overgrowth into the cartilage to be formed by the implanted chondrocytes. One of the types of commercial products which can be used in this invention is Surgicel® (Ethicon Ltd., UK) which is absorbable after a period of 7 - 14 days. The use of this material in the method of the instant invention is contrary to the normal use of a hemostatic device, such as Surgicel® as it is described in the package insert from Ethicon Ltd.

[0015] Surprisingly, we have found that in a situation where you wish to inhibit re-vascularization into cartilage, a hemostatic material will act like a gel-like artificial coagulate. If red blood cells should be present within the full-thickness defect of articular cartilage that is capped by such a hemostatic barrier, these blood cells will be chemically changed to hematin, and thus rendered unable to induce vascular growth. Thus a hemostatic product used as a re-vascularization inhibitory barrier with or without fibrin adhesives, such as for example the Surgicel®, is effective for the envisioned method as taught by the instant invention. Another part of this invention is the use of a cell-free component, that is used as a patch covering the defective area of the joint into which the cultured chondrocytes/cartilage are being transplanted, using autologous chondrocytes for the transplantation. The method of the invention also contemplates the use of suitable allogenic chondrocytes or xenogenic chondrocytes for the repair of a cartilage defect.

[0016] Thus the instant invention teaches methods for effective repair or treatment of cartilage defects in articular joint bone surfaces which comprises administering an agent or device to block vascular invasion into the cartilage site to be repaired, and also providing for a cell-free barrier which will isolate the repair site and keep transplanted cells in place. Thus the instant invention also provides for a kit comprising a hemostatic barrier component for insertion into the site to be repaired, such that there is effective inhibition of vascularization into the site to be repaired; and once the chondrocytes to be transplanted are placed into the site to be repaired, a cell-free semi-permeable barrier is capped over the repair site such that the transplanted chondrocytes are held in place, but are still able to gain access to nutrients.

[0017] Certain aspects of the invention have been exemplified using an in vitro system to study the behavior

of the chondrocytes when in contact with a certain product or a combination of certain products that inhibit the formation of vascular tissue. This in vitro testing predicts the ability of certain tested materials to inhibit vascularization, as will occur in vivo where capillary loops project into the cartilage being established during the process of autologous transplantation of chondrocytes into defects in the cartilage.

[0018] Suitable hemostatic products will be characterized by having the ability to inhibit the growth, or invasion of vascular tissue, osteocytes, fibroblasts etc. into the developing cartilage. A suitable hemostatic material will achieve the goal of the method of the instant invention in that vascular and cellular invasion into developing cartilage should be prevented in order to optimize the formation of cartilage and achieve repair of the full-thickness of any defects in the articular cartilage. Ideally, the hemostatic barrier will be stable for an extended period of time sufficient to allow for full cartilage repair, and then be able to be resorbed or otherwise broken down over time. One material identified as suitable is called Surgicel® W1912 (an absorbable hemostat containing oxidized regenerated sterile cellulose; Lot GG3DH, Ethicon Ltd. UK). Another example of a suitable material is BioGide® (a commercially available type I collagen matrix pad; Geistlich Söhne, Switzerland).

[0019] Suitable organic glue material can be found commercially, such as for example Tisseel® or Tissucol® (fibrin based adhesive; Immuno AG, Austria), Adhesive Protein (Cat. #A-2707, Sigma Chemical, USA), and Dow Corning Medical Adhesive B (Cat. #895-3, Dow Corning, USA).

[0020] The surgical instruments contemplated by the instant invention can be manufactured from metal and/or plastic suitable for making single-use disposable, or multi-use reusable surgical instruments. The cutting instrument may contain cutting teeth that are fully circular or flat, or anything in between. As cartilage is a relatively soft material it may be advantageous to manufacture hardened plastic cutting edges which will be able to sculpt cartilage without being able to damage bone. Such cutting instruments can be manufactured to incorporate openings for administration of fluid, suction removal of cutting debris and fluid, and fiber optic threads for illumination and visualization of the defect site.

[0021] Certain aspects of the instant invention may be better understood as illustrated by the following examples, which are meant by way of illustration and not limitation.

Example 1

[0022] In order for the Surgicel® to be used according to the invention for preventing development of blood vessels into autologous implanted cartilage or chondrocytes, Surgicel® was first treated with a fixative, such as glutaric aldehyde. Briefly, Surgicel® was treated with 0.6% glutaric aldehyde for 1 minute, followed by several

washings to eliminate glutaric aldehyde residues that may otherwise be toxic to tissue. Alternatively, the Surgicel® was treated with the fibrin adhesive called Tisseel® prior to treatment with glutaric aldehyde as described in Example 2. It was found that the Surgicel® fixated for instance with a fixative such as glutaric aldehyde, washed with sterile physiological saline (0.9%) and stored in refrigerator, does not dissolve for 1 to 2 months. Generally, Surgical is resorbed in a period between 7 and 14 days. This time would be too short, because a longer time is needed in preventing the development of blood vessels or vascularization as such from the bone structure into the implanted cartilage before the implanted chondrocytes have grown into a solid cartilage layer getting its nutrition requirements from the neighboring cartilage. In other words sufficient inhibition of the vascularization is needed for a longer time such as for instance one month. Therefore, the product should not be absorbed significantly prior to that time. On the other hand resorption is needed eventually. Hence, the organic material used as an inhibiting barrier shall have these capabilities, and it has been found that the Surgicel® treated in this manner provides that function.

Example 2

[0023] The Surgicel® was also coated with an organic glue, in this example the glue used was Tisseel® but others can also be used. This product, together with the Surgicel® produces a useable barrier for the particular purpose of the invention. Any other hemostat or vascular inhibiting barrier could be used. The Tisseel® was mixed as described below. The Surgicel® was then coated with Tisseel® by spraying the Surgicel® material on both sides until soaked. The Tisseel® (fibrin glue) was then allowed to solidify at room temperature. Immediately prior to completed solidification, the coated Surgicel® was then placed in 0.6% glutaric aldehyde for 1 minute and then washed with sterile physiological (0.9%) saline. The pH was then adjusted by PBS and/or with NaOH until pH was stable at 7.2 to 7.4. Afterwards the thus treated Surgicel® was then washed in tissue culture medium such as minimum essential medium/F 12 with 15 mM Hepes buffer.

[0024] As mentioned in this example we have used Tisseel® as the fibrin adhesive to coat the Surgicel®. Furthermore the fibrin adhesive or glue may also be applied directly on the bottom of the lesion towards the bone, on which the Surgicel® is glued. The *in vitro* system used, in lieu of *in vivo* testing, consisted of a NUNCLON™ Delta 6-well sterile disposable plate for cell research work (NUNC, InterMed, Roskilde, Denmark). Each well measures approximately 4 cm in diameter.

[0025] In the invention the fibrin adhesive can be any adhesive which together with the fibrin component will produce a glue that can be tolerated in humans (Ihara, N, et al., Burns Incl. Therm. Inj., 1984, 10, 396). The

invention also anticipates any other glue component that can be used in lieu of the fibrin adhesive. In this invention we used Tisseel® or Tissucol® (Immuno AG, Vienna, Austria). The Tisseel® kit consists of the following components:

Tisseel®, a lyophilized, virus-inactivated Sealer, containing clottable protein, thereof: fibrinogen, Plasma fibronectin (CIG) and Factor XIII, and Plasminogen.

Aprotinin Solution (bovine)

Thrombin 4 (bovine)

Thrombin 500 (bovine)

Calcium Chloride solution

[0026] The Tisseel® kit contains a DUPLOJECT® Application System. The fibrin adhesive or the two-component sealant using Tisseel® Kit is combined in the following manner according to the Immuno AG product insert sheet:

Example 3

[0027] Chondrocytes were grown in minimal essential culture medium containing HAM F12 and 15 mM Hepes buffer and 5 to 7.5% autologous serum in a CO₂ incubator at 37 °C and handled in a Class 100 laboratory at Verigen Europe A/S, Symbion Science Park, Copenhagen, Denmark. Other compositions of culture medium may be used for culturing the chondrocytes. The cells were trypsinized using trypsin EDTA for 5 to 10 minutes and counted using Trypan Blue viability staining in a Bürker-Türk chamber. The cell count was adjusted to 7.5 x 10⁵ cells per ml. One NUNCLON™ plate was uncovered in the Class 100 laboratory.

[0028] The Surgicel® hemostatic barrier was cut to a suitable size fitting into the bottom of the well in the NUNCLON™ tissue culture tray. In this case a circle, of a size of approximately 4 cm (but could be of any possible size) and placed under aseptic conditions on the bottom in well in a NUNCLON™ Delta 6-well sterile disposable plate for cell research work (NUNC, InterMed, Roskilde, Denmark). The hemostatic barrier to be placed on the bottom of the well was pre-treated as described in Example 1. This treatment delays the absorption of the Surgicel significantly. This hemostatic barrier was then washed several times in distilled water and subsequently several times until non-reacted glutaraldehyde was washed out. A small amount of the cell culture medium containing serum was applied to be absorbed into the hemostatic barrier and at the same time keeping the hemostatic barrier wet at the bottom of the well.

[0029] Approximately 10⁶ cells in 1 ml culture medium were placed directly on top of the hemostatic barrier, dispersed over the surface of the hemostatic barrier pre-treated with 0.4% glutaraldehyde as described above. The plate was then incubated in a CO₂ incubator at 37 °C for 60 minutes. An amount of 2 to 5 ml of tissue culture

medium containing 5 to 7.5% serum was carefully added to the well containing the cells avoiding splashing the cells by holding the pipette tip tangential to the side of the well when expelling the medium. It appeared that the pH of the medium was too low (pH ~6.8). The pH was then adjusted to 7.4 to 7.5. The next day some chondrocytes had started to grow on the hemostatic barrier, arranged in clusters. Some of the cells had died due to the low pH exposure prior to the adjustment of the pH. The plate was incubated for 3 to 7 days with medium change at day 3.

[0030] At the end of the incubation period the medium was decanted and cold refrigerated 2.5% glutaraldehyde containing 0.1M sodium salt of dimethylarsinic acid, (also called sodium cacodylate, pH is adjusted with HCl to 7.4), was added as fixative for preparation of the cell and supporter (hemostatic barrier) for later preparation for electron microscopy.

Example 4

[0031] Chondrocytes were grown in minimal essential culture medium containing HAM F12 and 15 mM Hepes buffer and 5 to 7.5% autologous serum in a CO₂ incubator at 37 °C and handled in a Class 100 laboratory at Verigen Europe A/S, Symbion Science Park, Copenhagen, Denmark. Other compositions of culture medium may be used for culturing the chondrocytes. The cells were trypsinized using trypsin EDTA for 5 to 10 minutes and counted using Trypan Blue viability staining in a Bürker-Türk chamber. The cell count was adjusted to 7.5 x 10⁵ cells per ml. One NUNCLON™ plate was uncovered in the Class 100 laboratory.

[0032] The Surgicel® (for use as the hemostatic barrier) was treated with 0.6% glutaric aldehyde for one minute as described in Example 1, and washed with 0.9% sterile sodium chloride solution or, preferably, with a buffer such as a PBS buffer or the culture medium such as MEM/F12, because pH after the glutaric aldehyde treatment is 6.8 and should preferably be 7.0 to 7.5. The Tisseel® was applied on both side of the Surgicel® using the DUPLOJECT® system, thus coating both sides of the Surgicel®, the patch intended to be used, with fibrin adhesive. The glue is left to dry under aseptic condition for at least 3 to 5 minutes. The "coated" hemostatic barrier was placed on the bottom of the well in a NUNCLON™ Delta 6-well sterile disposable plate for cell research work. A small amount of tissue culture medium containing serum was applied to be absorbed into the hemostatic barrier. Approximately 10⁶ cells in 1 ml tissue culture medium containing serum was placed directly on top of the Hemostat, dispersed over the surface of the hemostatic barrier. The plate was then incubated in a CO₂ incubator at 37 °C for 60 minutes. An amount of 2 to 5 ml of tissue culture medium containing 5 to 7.5 % serum was carefully added to the well containing the cells avoiding splashing the cells by holding the pipette tip tangential to the side of the well when expelling the

medium. After 3 to 6 days, microscopic examination showed that the cells were adhering to and growing into the Surgicel® in a satisfactory way suggesting that Surgicel® did not show toxicity to the chondrocytes and that the chondrocytes grew in a satisfactory manner into the Surgicel®.

[0033] The plate was incubated for 3 to 7 days with medium change at day 3. At the end of the incubation period the medium was decanted and cold refrigerated 2.5% glutaraldehyde containing 0.1M sodium salt of dimethylarsinic acid, also called sodium cacodylate, pH is adjusted with HCl to 7.4, was added as fixative for preparation of the cell and supporter (hemostatic barrier) for later preparation for electron microscopy.

Example 5

[0034] Chondrocytes were grown in minimal essential culture medium containing HAM F12 and 15 mM Hepes buffer and 5 to 7.5% autologous serum in a CO₂ incubator at 37 °C and handled in a Class 100 laboratory at Verigen Europe A/S, Symbion Science Park, Copenhagen, Denmark. The cells were trypsinized using trypsin EDTA for 5 to 10 minutes and counted using Trypan Blue viability staining in a Bürker-Türk chamber. The cell count was adjusted to 7.5 x 10⁵ to 2 x 10⁶ cells per ml. One NUNCLON™ plate was uncovered in the Class 100 laboratory.

[0035] It has been found that the Bio-Gide® can be used as a resorbable bilayer membrane which will be used as the patch or bandage covering the defective area of the joint into which the cultured chondrocytes are being transplanted as well as the hemostatic barrier. The Bio-Gide® is a pure collagen membrane obtained by standardized, controlled manufacturing processes (by E.D. Geistlich Söhne AG, CH-6110 Wolhusen). The collagen is extracted from veterinary certified pigs and is carefully purified to avoid antigenic reactions, and sterilized in double blisters by γ-irradiation. The bilayer membrane has a porous surface and a dense surface. The membrane is made of collagen type I and type III without further cross-linking or chemical treatment. The collagen is resorbed within 24 weeks. The membrane retains its structural integrity even when wet and it can be fixed by sutures or nails. The membrane may also be "glued" using fibrin adhesive such as Tisseel® to the neighboring cartilage or tissue either instead of sutures or together with sutures.

[0036] The Bio-Gide® was un-covered in a class 100 laboratory and placed under aseptic conditions on the bottom of the wells in a NUNCLON™ Delta 6-well sterile disposable plate for cell research work, - either with the porous surface of the bilayer membrane facing up or with the dense surface facing up. Approximately 10⁶ cells in 1 ml tissue culture medium containing serum was placed directly on top of the Bio-Gide®, dispersed either over the porous or the dense surface of the Bio-Gide®. The plate was then incubated in a CO₂ incubator

at 37 °C for 60 minutes. An amount of 2 to 5 ml of tissue culture medium containing 5 to 7.5 % serum was carefully added to the well containing the cells avoiding splashing the cells by holding the pipette tip tangential to the side of the well when expelling the medium.

[0037] On day 2 after the chondrocytes were placed in the well containing the Bio-Gide® the cells were examined in a Nikon Inverted microscope. It was noticed that some chondrocytes had adhered to the edge of the Bio-Gide. It was of course not possible to be able to look through the Bio-Gide® itself using this microscope.

[0038] The plate was incubated for 3 to 7 days with medium change at day 3. At the end of the incubation period the medium was decanted and cold refrigerated 2.5% glutaraldehyde containing 0.1M sodium salt of dimethylarsinic acid, also called sodium cacodylate; pH is adjusted with HCl to 7.4, was added as fixative for preparation of the cell and the Bio-Gide® supporter with the cells either cultured on the porous surface or the dense surface. The Bio-Gide® patches were then sent for electron microscopy at Department of Pathology, Herlev Hospital, Denmark.

[0039] The electron microscopy showed that the chondrocytes cultured on the dense surface of the Bio-Gide® did not grow into the collagen structure of the Bio-Gide®, whereas the cells cultured on the porous surface did indeed grow into the collagen structure and furthermore, showed presence of proteoglycans and no signs of fibroblast structures. This result shows that when the collagen patch, as for instance a Bio-Gide® patch is sewn as a patch covering a cartilage defect the porous surface shall be facing down towards the defect in which the cultured chondrocytes are to be injected. They will then be able to penetrate the collagen and produce a smooth cartilage surface in line with the intact surface, and in this area a smooth layer of proteoglycans will be built up. Whereas, if the dense surface of the collagen is facing down into the defect the chondrocytes to be implanted will not integrate with the collagen, and the cells will not produce the same smooth surface as described above.

Example 6

[0040] Chondrocytes were grown in minimal essential culture medium containing HAM F12 and 15 mM HEPES buffer and 5 to 7.5% autologous serum in a CO₂ incubator at 37 °C and handled in a Class 100 laboratory at Verigen Europe A/S, Symbion Science Park, Copenhagen, Denmark. The cells were trypsinized using trypsin EDTA for 5 to 10 minutes and counted using Trypan Blue viability staining in a Bürker-Türk chamber. The cell count was adjusted to 7.5×10^5 to 2×10^6 cells per ml. One NUNCLON™ plate was uncovered in the Class 100 laboratory.

[0041] The Bio-Gide® used as a resorbable bilayer membrane may also be used together with an organic glue such as Tisseel® with additional, significantly high-

er content of Aprotinin than normally found in Tisseel®, as described in the product insert. By increasing the content of Aprotinin to about 25,000 KIU/ml, the resorption of the material will be delayed by weeks instead of the normal span of days.

[0042] To test this feature in vitro, the Tisseel® is applied to the bottom of the well of the NUNCLON™ plate, and allowed to solidify incompletely. A collagen patch such as a Bio-Gide® is then applied over the Tisseel® and glued to the bottom of the well. This combination of Bio-Gide® and Tisseel® is designed to be a hemostatic barrier that will inhibit or prevent development or infiltration of blood vessels into the chondrocyte transplantation area. This hybrid collagen patch can now be used for both as a hemostatic barrier at the bottom of the lesion (most proximal to the surface to be repaired) but also as a support for cartilage formation because the distal surface can be the porous side of the collagen patch and thus encourage infiltration of chondrocytes and cartilage matrix. Thus this hybrid collagen patch can also be used to cover the top of the implant with the collagen porous surface directed down towards the implanted chondrocytes and the barrier forming the top. The hybrid collagen patch, with elevated Aprotinin component may also be used without any organic glue such as Tisseel® and placed within the defect directly, adhering by natural forces. Thus the collagen patch can be used both as the hemostatic barrier, and the cell-free covering of the repair/transplant site, with the porous surfaces of the patches oriented towards the transplanted chondrocytes/cartilage. Another variant would use a collagen patch which consists of type II collagen (ie. from Geistlich Sohne AG, CH-6110 Wolhusen).

[0043] Thus the instant invention provides for a hybrid collagen patch where said patch is a collagen matrix with elevated levels of aprotinin component, preferably about 25,000 KIU/ml, in association with an organic matrix glue, where the collagen component is similar to the Bio-Gide® resorbable bilayer material or Type II collagen, and the organic glue is similar to the Tisseel® material. In another embodiment, the hybrid collagen patch does not use any organic glue to adhere to the site of the repair.

Example 7

[0044] Because of the weakened structure of osteoarthritic cartilage, adherence of cultured autologous chondrocytes transplanted to a graft site in defective cartilage may be inhibited, thus creating a marginal zone (zone of demarcation) between the newly implanted cartilage/chondrocytes and the surrounding established cartilage. This marginal zone will be most pronounced if the graft site is prepared for the graft by creating straight, smooth walls cut in a linear fashion. The shearing and compression forces across such a marginal zone (as illustrated in Figure 3A) will exert great force to dislodge the graft when the graft site is cut in a linear

fashion. This marginal zone, and differential movement of materials along this zone will inhibit confluent healing between the grafted material and the surrounding material. This marginal zone shearing is exacerbated when the hardness of the abutting material is different. In many cases the graft material is softer than the surrounding material, however, in some instances of osteoarthritis disease, the surrounding cartilage may in fact be softer than the implanted chondrocytes/cartilage.

[0045] Therefore, in order to solve this problem, the method of the invention teaches the use of surgical instruments to sculpt the walls of the graft site such that the walls are non-linear, and thus provide for undulated surfaces which will reduce marginal zone shearing and provide anchorage for grafted material. It is also possible to shape the graft site such that the diameter of the site proximal to the bone surface is of a greater dimension than the opening distal to the bone and at the surface of the cartilage to be repaired such that there is a "reverse funnel" effect. A narrowed opening at the surface will aid in reducing marginal zone shearing and the expulsion of graft material from the graft site. A preferred embodiment describes the sculpting of the walls of the graft site in a fashion similar to a threaded opening for receiving a bolt or screw (as illustrated in Figure 3B), thus providing mechanical resistance to the compression and or ejection of the grafted material from the graft site which can be described as "male" and "female" threading.

[0046] The surgical instruments contemplated by the instant invention can be manufactured from metal and/or plastic suitable for making single-use disposable, or multi-use reusable surgical instruments. As cartilage is a relatively soft material it may be advantageous to manufacture hardened plastic cutting edges which will be able to sculpt cartilage without being able to damage bone. Such cutting instruments can be manufactured to incorporate openings for administration of fluid, suction removal of cutting debris and fluid, and fiber optic threads for illumination and visualization of the defect site. In certain embodiments of the instrument, the base of the instrument may have protruding point or pin-like structure which will assist in guiding and placing the instrument in the graft site. Of course such a pin would be designed to minimized damage to the underlying bone.

[0047] While the cutting surface of the instrument may be single toothed, or multi-toothed, or describe a screw-like pattern such as that in a metal tap used to generate threaded holes in metal parts, the characteristic required of the cutting instrument is that the resulting sculpted sides of the graft site is undulated, and non-linear. For example, in certain embodiments, the cutting edge of the instrument can be shaped similar to that shown in Figure 4A, or as in Figure 4B. The cutting edge maybe flat, or circular in that it wraps around the diameter of the cutting instrument. Many other shapes can be designed to accomplish the purpose of the method of the invention to create an interface which provides for

mechanical resistance to differential reaction to compression and shearing forces on the transplanted material and the surrounding material.

5 Example 8

[0048] A four month old mixed Yorkshire breed pig was subjected to general anesthesia and placed on its back. The pig was washed and draped in a surgical suite at Harrington Arthritis Research Center, Phoenix, Arizona. The entire surgical procedure was performed aseptically. The left hind-leg and adjacent abdomen and inguinal area was cleaned with iodine. The knee joint was localized, and the patella localized. A medial incision was performed approximately 3 cm from the posterior part of the patella and the several subcutis, muscle layers and ligaments was cut approximately in order to get access to the medial femoral condyle. Using a circular cutter a lesion was prepared in the white cartilage on the medial part of the medial condyle, leaving a 0.5 to 1 cm margin to the edge of the cartilage covering the posterior-medial part of the condyle (left condyle, Figure 6A). The 0.5 to 1 cm defect was placed in a caudal weight bearing part of the medial condyle. The entire surgical procedure was done without tourniquet on the left femur. The different layers and skin was sutured appropriately.

[0049] On day 3 the animal was again brought to the surgical suite and positioned as above on the operating table and given general anesthesia. The left hind leg, abdomen and inguinal region was ionized as described above. Sutures were cut and the area opened. It was noticed that a moderate hematoma was present in the knee joint. The blood clot was removed and the defect inspected. There was a blood clot in the defect which was removed. A sterile surgical instrument designed with a male thread cutting edge, with a size corresponding to, or slightly bigger than the circumference of the lesion was carefully screwed down into the defect. A BioGide® pad was cut to a size equal to the bottom of the defect. The first glue used, called Adhesive Protein (A-2707, Sigma Chemical, USA) was applied on the dense side of the trimmed hemostatic barrier pad, and the pad was placed dense side down into the bottom of the lesion, using it as a barrier as described above. It was found that this glue did not seem to dry very fast. The slight bleeding from the bottom of the defect stopped immediately. A second BioGide® was cut somewhat bigger in circumference than the lesion and was placed with dense side up (thus the porous side down towards the graft) as described above.

[0050] This non-cellular covering-pad was then sutured over the cavity, leaving one edge open, where the chondrocyte to be explanted could be injected into the graft site. The surrounding part of the edge of the pad was covered with the second glue, Dow Corning Medical Adhesive B (Cat. #895-3, Dow Corning, USA). This second glue dried much faster and more efficiently than

the first glue. It was found that during this particular procedure, the first glue had not dried sufficiently to hold the hemostatic barrier in place when suturing of the cap was attempted. The main barrier formed on the proximal surface of the graft site was by the glue itself.

[0051] Using a 1 ml syringe and a 16 gauge needle, the chondrocyte cell suspension (about 0.6 ml) was drawn up into the barrel of the syringe. A 23 gauge short needle was switched for the 16 gauge needle, and the cell suspension was injected under the sutured covering-patch into the graft site (about 10×10^6 cells). The open edge of the cap was then glued prior to removal of the needle, and the needle carefully withdrawn. No leakage of cells was seen. The wound was sutured and as above, no tourniquet was used, no bleeding was observed. The final skin layers were sutured. No protrusion of the skin occurred after suturing, which indicates that there was no hematoma. Postoperative recovery was uneventful.

[0052] As expected, the grafted chondrocytes produced cartilage matrix sufficient to repair the defect made in the articular cartilage surface of the knee joint of the test pig. Figure 6A is an MRI image of a pig knee showing the cartilage defect created in the knee (left condyle, the medial condyle), and Figure 6B is an MRI image of the same pig knee three months after treatment showing repair of the defect.

Example 9

[0053] A kit comprising the components useful for practicing the method of the invention, will allow for the convenient practice of the method of the invention in a surgical setting. In a preferred embodiment, a kit of the invention will provide sterile components suitable for easy use in the surgical environment, and will provide a suitable hemostatic barrier, suitable covering patch, and if needed organic glue. A kit of the invention may also provide sterile, cell-free matrix material suitable for supporting autologous chondrocytes that are to be implanted into an articular joint surface defect. In one embodiment, a kit of the invention contains a Surgicel® hemostatic barrier and a Bio-Gide® covering patch with suitable coating of Tisseel® organic glue, where the Surgicel® and Bio-Gide® have been treated according to the teachings of the invention to increase the time till resorption. In instances where Tisseel® is pre-coated, in one embodiment the Tisseel® is supplemented with additional aprotinin to increase time till resorption.

[0054] In another preferred embodiment, the hemostatic barrier and covering-patch are both a semi-permeable collagen matrix which is treated to extend the time till resorption of the material. It is also possible to provide Tisseel® glue in enhanced form as a separate component to be applied as needed because of the inherent variability and unique circumstances every repair/transplantation procedure will encounter.

[0055] A further embodiment of a kit of the invention

will include a surgical instrument as described in Example 7 above, or suitable variations thereof.

[0056] It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention shown in the specific embodiments without departing from the spirit and scope of the invention as described.

Claims

1. A cartilage repair structure for repair of a defect in articular cartilage comprising:
 - a. a cell free component having a porous surface; and
 - b. chondrocytes adjacent said porous surface of said cell-free component,
2. The cartilage repair structure according to claim 1, wherein said chondrocyte cells are adhered to said porous surface.
3. The cartilage repair structure according to claims 1 and 2, wherein said component is collagen,
4. The cartilage repair structure according to claims 1 to 3, wherein said component is Type I and Type III collagen.
5. The cartilage repair structure according to claims 1 to 4, wherein said component is resorbable.
6. The cartilage repair structure according to claims 1 to 5, wherein said chondrocyte cells are autologous, allogenic, and/or xenogenic.
7. The cartilage repair structure according to claims 1 to 6, further comprising a biocompatible adhesive adjacent said component.
8. The cartilage repair structure according to claims 1 to 7, wherein said component is adapted to be disposed over an articular cartilage defect.
9. The cartilage repair structure according to claims 1 to 7, wherein said component is adapted to be disposed in an articular cartilage defect.

FIG. 1A

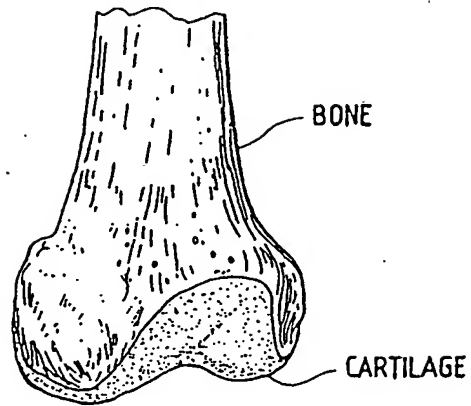


FIG. 1B

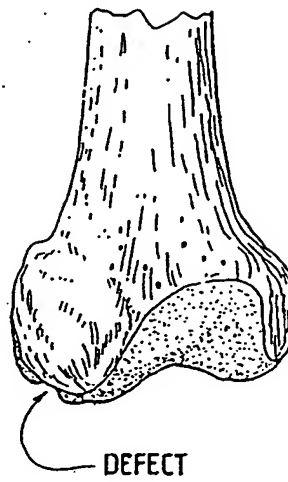


FIG. 1C

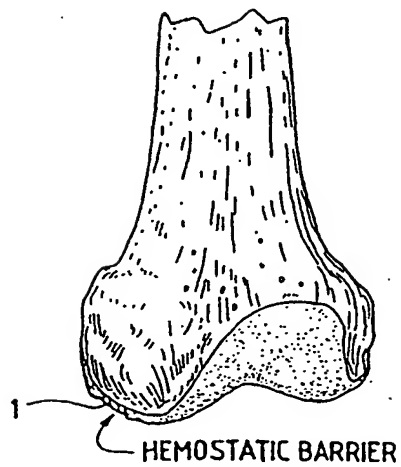


FIG. 2

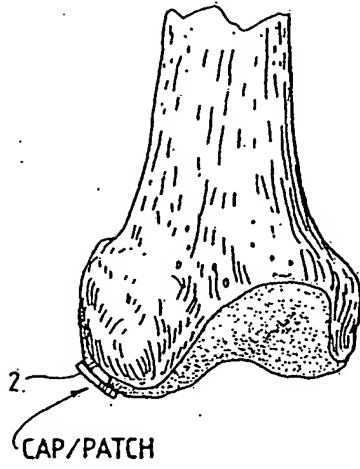


FIG. 3A

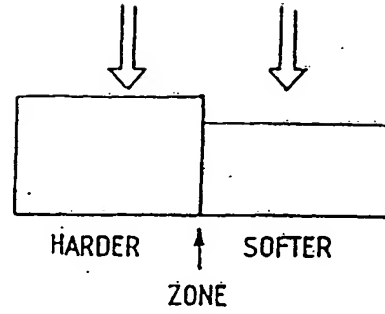


FIG. 3B

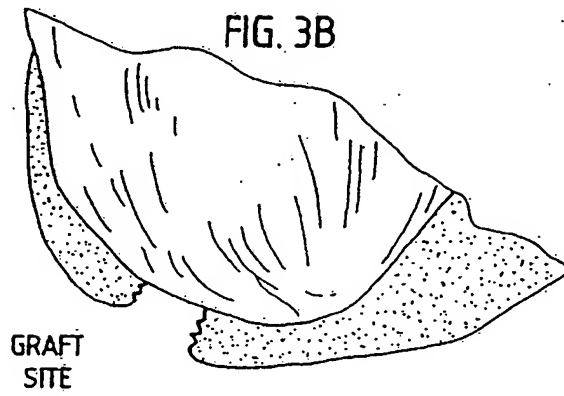


FIG. 3C

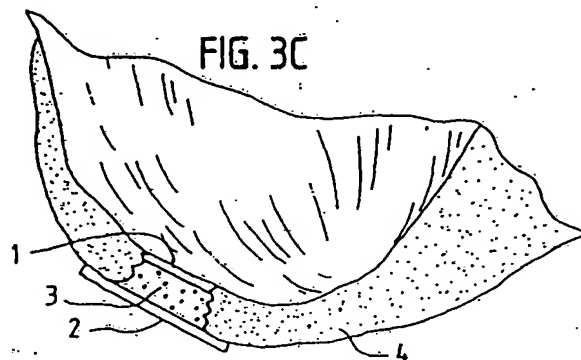


FIG. 4A

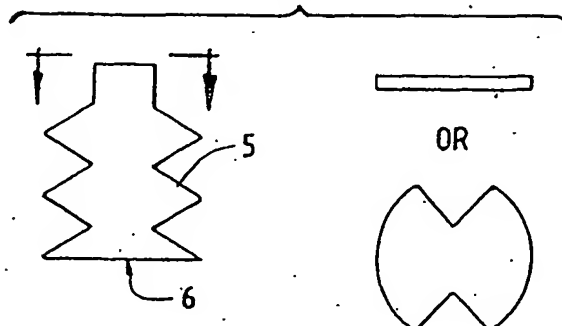


FIG. 4B

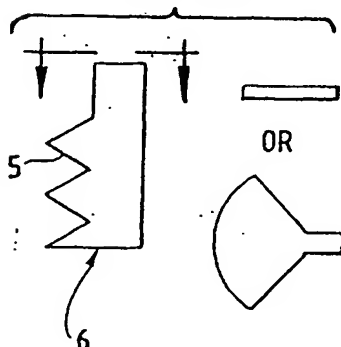


FIG. 5

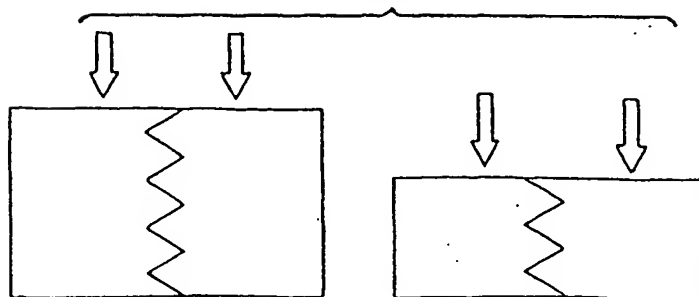




FIG. 6A

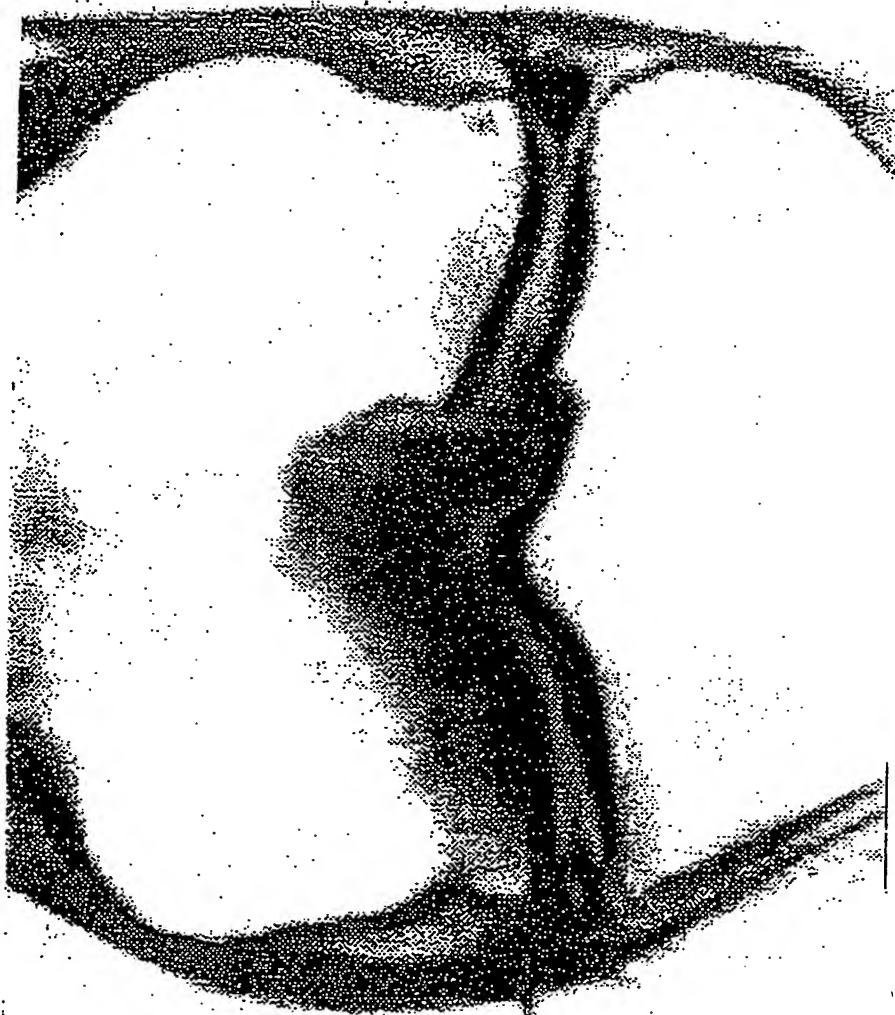


Fig. 6b



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 03 02 3272

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (In I.C.I.6)
A	US 4 846 835 A (GRANDE) 11 July 1989 (1989-07-11) * abstract; claims 1,4 *	1,3,4	A61F2/30 A61L27/00 A61B17/32
A	WO 95 30742 A (BIOSURFACE TECHNOLOGY) 16 November 1995 (1995-11-16) * abstract; claim 21 *	1,3,4	
A	WO 96 24310 A (THE HOSPITAL FOR JOINT DISEASES, ORTHOPAEDIC INSTITUTE) 15 August 1996 (1996-08-15) * the whole document *	1,3,4	
A	GB 2 137 209 A (RAMOT UNIVERSITY AUTHORITY FOR APPLIED RESEARCH AND INDUSTRIAL DEVELOP) 3 October 1984 (1984-10-03) * the whole document *	1	
A	EP 0 339 607 A (ITAY) 2 November 1989 (1989-11-02) * the whole document *	1	
A	WO 94 09722 A (THM BIOMEDICAL) 11 May 1994 (1994-05-11)		TECHNICAL FIELDS SEARCHED (In I.C.I.6) A61F A61B
A	WO 93 11723 A (REGEN CORPORATION) 24 June 1993 (1993-06-24)		
A	EP 0 530 804 A (SHAW) 10 March 1993 (1993-03-10)		
A	WO 90 12603 A (VACANTI) 1 November 1990 (1990-11-01)		
A	WO 92 13565 A (SHAW) 20 August 1992 (1992-08-20)		
		-/--	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 2 December 2003	Examiner Klein, C
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

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European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 03 02 3272

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	WO 93 15694 A (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 19 August 1993 (1993-08-19) ---		
A	WO 94 26211 A (GROSS) 24 November 1994 (1994-11-24) ---		
A	EP 0 277 678 A (STICHTING SCIENCE PARK GRONINGEN) 10 August 1988 (1988-08-10) -----		
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 2 December 2003	Examiner Klein, C
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : member of the same patent family, corresponding document X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document	

EPO FORM 1503 (03.02.02) (P04C01)

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 03 02 3272

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on the European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

02-12-2003

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 4846835	A	11-07-1989	NONE	
WO 9530742	A	16-11-1995	AU 2435495 A	29-11-1995
			AU 2468995 A	29-11-1995
			WO 9530383 A1	16-11-1995
			WO 9530742 A1	16-11-1995
			US 5723331 A	03-03-1998
			US 5786217 A	28-07-1998
WO 9624310	A	15-08-1996	US 6080194 A	27-06-2000
			AU 693323 B2	25-06-1998
			AU 4918996 A	27-08-1996
			CA 2212632 A1	15-08-1996
			DE 69629666 D1	02-10-2003
			EP 0808142 A1	26-11-1997
			JP 10513386 T	22-12-1998
			WO 9624310 A1	15-08-1996
GB 2137209	A	03-10-1984	IL 68218 A	31-12-1985
			DE 3410631 A1	27-09-1984
			JP 1839264 C	25-04-1994
			JP 5051306 B	02-08-1993
			JP 59192364 A	31-10-1984
			US 4642120 A	10-02-1987
EP 339607	A	02-11-1989	US 5053050 A	01-10-1991
			US 4904259 A	27-02-1990
			AT 85524 T	15-02-1993
			AU 625913 B2	16-07-1992
			AU 3380889 A	02-11-1989
			DE 68904800 D1	25-03-1993
			DE 68904800 T2	05-08-1993
			EP 0339607 A2	02-11-1989
			ES 2051914 T3	01-07-1994
			IL 90075 A	26-08-1994
			JP 2209811 A	21-08-1990
			JP 2858782 B2	17-02-1999
			ZA 8903074 A	29-05-1991
WO 9409722	A	11-05-1994	AU 5445794 A	24-05-1994
			BR 9307280 A	01-06-1999
			CA 2147359 A1	11-05-1994
			GB 2215209 A ,B	20-09-1989
			JP 1232967 A	18-09-1989
			JP 2820415 B2	05-11-1998
			WO 9409722 A1	11-05-1994

EPO FORM P0458

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 03 02 3272

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

02-12-2003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9409722 A		US 5683459 A	04-11-1997
		US 5904717 A	18-05-1999
		US 6005161 A	21-12-1999
		US 5755792 A	26-05-1998
		US 5133755 A	28-07-1992
		US 5366508 A	22-11-1994
WO 9311723 A	24-06-1993	US 5306311 A	26-04-1994
		AU 669686 B2	20-06-1996
		AU 3229193 A	19-07-1993
		CA 2125967 A1	24-06-1993
		EP 0617598 A1	05-10-1994
		JP 7505792 T	29-06-1995
		US 6042610 A	28-03-2000
		WO 9311723 A1	24-06-1993
		US 5624463 A	29-04-1997
		US 5681353 A	28-10-1997
		US 5735903 A	07-04-1998
		US 5735902 A	07-04-1998
EP 530804 A	10-03-1993	US 5270300 A	14-12-1993
		AU 657888 B2	23-03-1995
		AU 2541192 A	05-04-1993
		CA 2116859 A1	18-03-1993
		EP 0530804 A1	10-03-1993
		IL 102988 A	08-02-1998
		JP 7500741 T	26-01-1995
		NO 940764 A	29-04-1994
		NZ 244060 A	27-07-1997
		TW 436300 B	28-05-2001
		WO 9304710 A2	18-03-1993
		ZA 9206729 A	12-03-1993
WO 9012603 A	01-11-1990	US 5041138 A	20-08-1991
		AT 142511 T	15-09-1996
		AU 635025 B2	11-03-1993
		AU 5556890 A	16-11-1990
		CA 2051663 A1	18-10-1990
		DE 69028524 D1	17-10-1996
		DE 69028524 T2	13-02-1997
		DK 469070 T3	30-09-1996
		EP 0469070 A1	05-02-1992
		ES 2095252 T3	16-02-1997
		JP 6006155 B	26-01-1994
		JP 4505717 T	08-10-1992
		WO 9012603 A1	01-11-1990

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 03 02 3272

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

02-12-2003

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9012603	A		US 5736372 A	07-04-1998
WO 9213565	A	20-08-1992	US 5206023 A	27-04-1993
			AT 121943 T	15-05-1995
			AU 667032 B2	07-03-1996
			AU 1412892 A	07-09-1992
			CA 2101556 A1	01-08-1992
			CN 1064813 A ,B	30-09-1992
			DE 69202332 D1	08-06-1995
			DE 69202332 T2	04-01-1996
			DK 569541 T3	10-07-1995
			EP 0569541 A1	18-11-1993
			ES 2072144 T3	01-07-1995
			HK 1006416 A1	26-02-1999
			IE 920309 A1	29-07-1992
			IL 100799 A	16-10-1996
			JP 6505258 T	16-06-1994
			KR 235391 B1	15-12-1999
			NO 932748 A	29-09-1993
			NZ 260125 A	27-07-1997
			TW 505518 B	11-10-2002
			WO 9213565 A1	20-08-1992
			US 5853746 A	29-12-1998
			US 5368858 A	29-11-1994
			ZA 9200726 A	25-11-1992
WO 9315694	A	19-08-1993	AU 3619293 A	03-09-1993
			CA 2117379 A1	19-08-1993
			DE 69307299 D1	20-02-1997
			EP 0625891 A1	30-11-1994
			HK 71597 A	06-06-1997
			JP 7503869 T	27-04-1995
			WO 9315694 A1	19-08-1993
			US 5607474 A	04-03-1997
			US 6013853 A	11-01-2000
			US 5876452 A	02-03-1999
WO 9426211	A	24-11-1994	DE 4317448 A1	24-11-1994
			AU 6792994 A	12-12-1994
			WO 9426211 A1	24-11-1994
EP 277678	A	10-08-1988	NL 8700113 A	16-08-1988
			AU 1037688 A	21-07-1988
			DK 20988 A	20-07-1988
			EP 0277678 A1	10-08-1988
			FI 880147 A	20-07-1988

EPO FORM P0058

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

